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SPECIAL REPORT # 98

AEROBIOLOGICAL STUDIES: SONIC-SPEED, LIQUID IMPINGER FOR COLLECTING SAMPLES OF MICROBIOLOGICAL MATERIALS FROM AEROSOLS

CAMP DETRICK; FREDERICK, MARYLAND
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TABLE OF CONTENTS

AEROBIOLOGICAL STUDIES: SONIC-SPEED, LIQUID IMPINGER FOR COLLECTING SAMPLES OF MICROBIOLOGICAL MATERIALS FROM AEROSOLS

TABLES

[redacted]

ILLUSTRATIONS

[redacted]

AEROBIOLOGICAL STUDIES:

SONIC-SPEED, LIQUID IMPINGER FOR COLLECTING SAMPLES OF MICROBIOLOGICAL MATERIALS FROM AEROSOLS

Special Report No. 98
Camp Detrick, Maryland

I. INTRODUCTION

The acquisition of a sampler or samplers, for use in determinations of the concentration, in aerosols, of such widely varied materials as bacteria, viruses, toxins, and dye tracers was one of the first problems of the Aerobiology Branch (formerly Cloud-Chamber Project) 1/. This Project was approved by letter of transmittal dated 3 November 1943, Chief of the Chemical Warfare Service. Most of the experimental work on sampling was done under Project Specification Number C8.2 during the period 1945-1947.

Among the specifications considered for the desired sampler were:

1. The sampler should be suitable for efficiently collecting samples of such varied materials as bacteria, viruses, toxins, and dye tracers over a wide range of concentrations.

2. The sample collected must be suitable for quantitating; that is, the material must not be destroyed during or after collection and it must not be retained by the sampler so that it is not available for quantitating.

3. The sampler should be such that it could be readily sterilized or decontaminated.

4. The sampler should require only a small portion of the flow or volume of atmosphere being studied.

5. The sampling rate should be fixed, or easy to regulate.

6. The sampler should be small, available, and economical enough to permit collection of many (at least 25) samples in a single experiment.

7. The sampler should have a record of successful use.

1/ Rosebury, T., et al, 1947, Experimental Air-borne Infection: Williams & Wilkins Co., Baltimore, Md., 222 pp.

[redacted]

[redacted]

Many samplers had been used or proposed for use in sampling air-borne bacteria. Among such samplers, arranged according to their main operating principle, were: (1) Sedimentation samplers- open Petri dish and Wells air centrifuge 2/; (2) filtration samplers- sugar tube or aeroscope 3/, sand tube 4/, cotton filter, and filter paper 5/; (3) washing samplers- water aeroscope 6/, bead bubbler 7/, atomizer bubbler 8/, and Folin-Lomon bubbler 9/; (4) impingement samplers- Greenburg-Smith impinger 5/ Porton impinger 10/ 11/, funnel device 12/, slit sampler 13/, and sieve device 14/

2/ Wells, W.F., 1933, Apparatus for the Study of the Bacterial Behavior of Air: Am. J. Pub. Health, 23, pp. 58-59.

3/ Frankland, P.F., 1886, A New Method for the Quantitative Estimation of the Microorganisms Present in the Atmosphere: Roy. Soc. London, Phil. Trans., Ser. B., 178, pp. 113-152.

4/ American Public Health Association, 1917, Final Report of Committee on Standard Methods for the Examination of Air: Am. J. Pub. Health, 7, p. 54.

5/ McConnell, W.J., and Thomas, B.G.H., 1925, Relative Values of Methods of Enumerating Bacteria in Air: Pub. Health Repts., 40, pp. 2167-2178.

6/ Rettger, L.F., 1910, Rettger Method of Determining Bacteria in the Air, Using Salt Solution: J. Med. Research, 22, pp. 461-468.

7/ Wheeler, S.M., Foley, G.E., and Jones, T.D., 1941, A Bubbler Pump Method for Quantitative Estimations of Bacteria in the Air: Science, 94, p. 445.

8/ Moulton, S., Puck, T.T., and Lemon, H.M., 1943, An Apparatus for Determination of the Bacterial Content of Air: Science, 97, p. 51.

9/ Lemon, H.M., 1943, A Method for Collection of Bacteria from Air and Textiles: Proc. Soc. Exptl. Biol. and Med., 54, pp. 298-301.

10/ Henderson, D.W., and Woods, D.D., 1942, A Laboratory Apparatus for Setting Up Bacterial Clouds with a Report on Experiments with B. Anthracis: Camp Detrick Tech. Library No. 3785.

11/ Sampling Devices: Impingers, from Dr. Fildes' "Bible", 1943, Camp Detrick Tech. Library No. 3784.

12/ Hollaender, A., and DallaValle, J.M., 1939, A Simple Device for Sampling Air-borne Bacteria: Pub. Health Repts., 54, pp. 574-577.

13/ Bourdillon, R.B., Lidwell, O.M., and Thomas, J.C., 1941, A Slit Sampler for Collecting and Counting Air-borne Bacteria: J. Hyg., 41, pp. 197-224.

14/ du Buy, H.G., and Crisp, L.R., 1944, A Sieve Device for Sampling Air-borne Microorganisms: Pub. Health Repts., 59, pp. 829-832.

[redacted]

and (5) electrostatic samplers- Berry funnel precipitator 15/, Barnes-Penney dust count 16/ and weight 17/ samplers, and G.E. electrostatic sampler 18/.

Most of these samplers failed to meet one or more of the preceding specifications for more or less obvious reasons. The open Petri dish sampler collected samples of the material that settled out of the air rather than of the material in the air. The Wells air centrifuge and the electrostatic samplers were too bulky for use in the available space; too complicated and sensitive for ready sterilization, especially by steam; and too expensive and of questionable availability in the number needed. The sugar in the sugar tube could not be autoclaved readily. The sand tube, cotton filter, filter paper, and other dry filters did not seem suitable because of the possible harmful effect of air passing over collected bacteria and difficulty in removing collected material from the sampler. The atomizer bubbler was rather fragile. The funnel device, slit sampler, and sieve device were designed for collection of bacteria on an agar or solid media surface 19/, thus they were suitable only for the collection of bacteria forming countable colonies.

The Porton impinger, a sonic-speed, liquid impinger, which had been developed by British investigators for sampling microbiological materials in aerosols, seemed to comply best with the preceding specifications. This impinger was a slight modification of impingers that had been in general use in the United States since 1922 by industrial hygienists for collecting samples of unhygienic dusts from aerosols 20/. The suitability of this sampler was investigated. The main changes made in the device were:

15/ Berry, C.M., 1941, An Electrostatic Method for Collecting Bacteria from Air: Pub. Health Repts., 56, pp. 2044-2051.

16/ Barnes, E.C., and Penney, G.W., 1936, An Electrostatic Dust Count Sampler: J. Ind. Hyg. Toxicol., 18, pp. 167-172.

17/ Barnes, E.C., and Penney, G.W., 1938, An Electrostatic Dust Weight Sampler; J. Ind. Hyg. Toxicol., 20, pp. 259-265.

18/ Luckiesh, M., et al , 1946, Sampling Air for Bacterial Content; New Mechanical Designs and Applications of Electrostatic Fields: Gen. Elec. Rev., 49, pp. 8-17.

19/ Schneiter, R., Dunn, J.E., and Caminita, B.H., 1945, Studies in Connection with the Selection of a Satisfactory Culture Medium for Bacterial Air Sampling: Pub. Health Repts., 60, pp. 789-806.

20/ Brown, C.E., and Schrenk, H.H., 1938, A Technique for Use of the Impinger Method: U.S. Bureau of Mines, Information Circular 7026, 20 pp.

(1) Substitution of readily-available 125 ml. filtering flasks for the straight-wall tube or bottle-type containers and (2) regulation and measurement of the sampling flow rate by drawing the maximum flow through the orifice instead of by maintaining a constant suction on the impinger.

This report contains information on the design of the final device adopted and quantitative data on such operating characteristics as: (1) Retention of collected material in the intake tube, (2) effect of impinging distance on sampling efficiency, (3) effect of sampling flows on sampling efficiency, and (4) sampling efficiency.

Sampling efficiency, as used in this report, is the ratio, expressed in per cent, of the material determined to the material actually present in the aerosol sampled. Thus,

$$E = 100\ C'/C \qquad (1)$$

in which E = sampling efficiency, C' = concentration of material found, and C = concentration of material actually present in the air. This differs from collecting efficiency in that all material collected is not necessarily determined. The sampling efficiency of many inanimate materials may be close to that of the collecting efficiency, but the sampling efficiency of animate materials such as bacteria may be much lower than that of the collecting efficiency because of loss of bacteria during or after sampling.

Some of the information in this report has been published in a monograph on experimental air-borne infection 21/.

The information has been obtained at irregular intervals since the start of the aerobiological program about December 1943.

II. DESCRIPTION OF IMPINGER

The final model of the impinger is shown in Figure 1. The container is a 125-ml. filtering flask, selected because of availability, ruggedness, stability in resting on a surface, and possession of a side arm. The intake tube is a tube 1 cm. in diameter having a piece of capillary tubing 1 cm. long sealed to its lower end. Such tubes can be made easily in the laboratory or can be obtained from such companies as Fischer & Porter at Hatboro, Pa. The upper end of the intake tube is bent at an angle of 45° to prevent material from dropping into the tube. The diameter of the capillary tubing used is one through which the maximum or limiting flow is equal or close to the desired sampling flow. Approximate orifice diameters for maximum or sampling flows from 1.3 to 12 liters per minute are given in Figure 2. Sampling flows of about 2.5 liters per minute have been the ones most used.

21/ Rosebury, T., et al , See footnote 1.

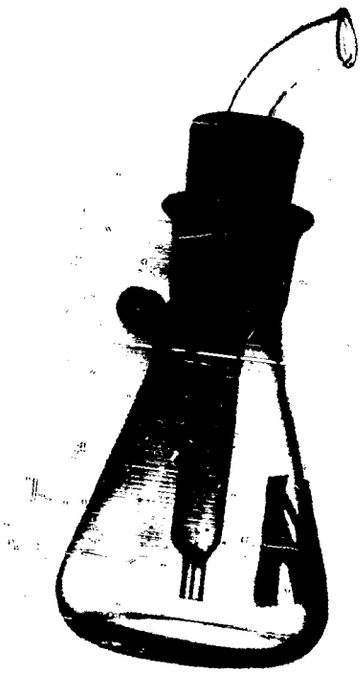

FIGURE 1

The sonic-speed, liquid impinger. Negative No. 7016

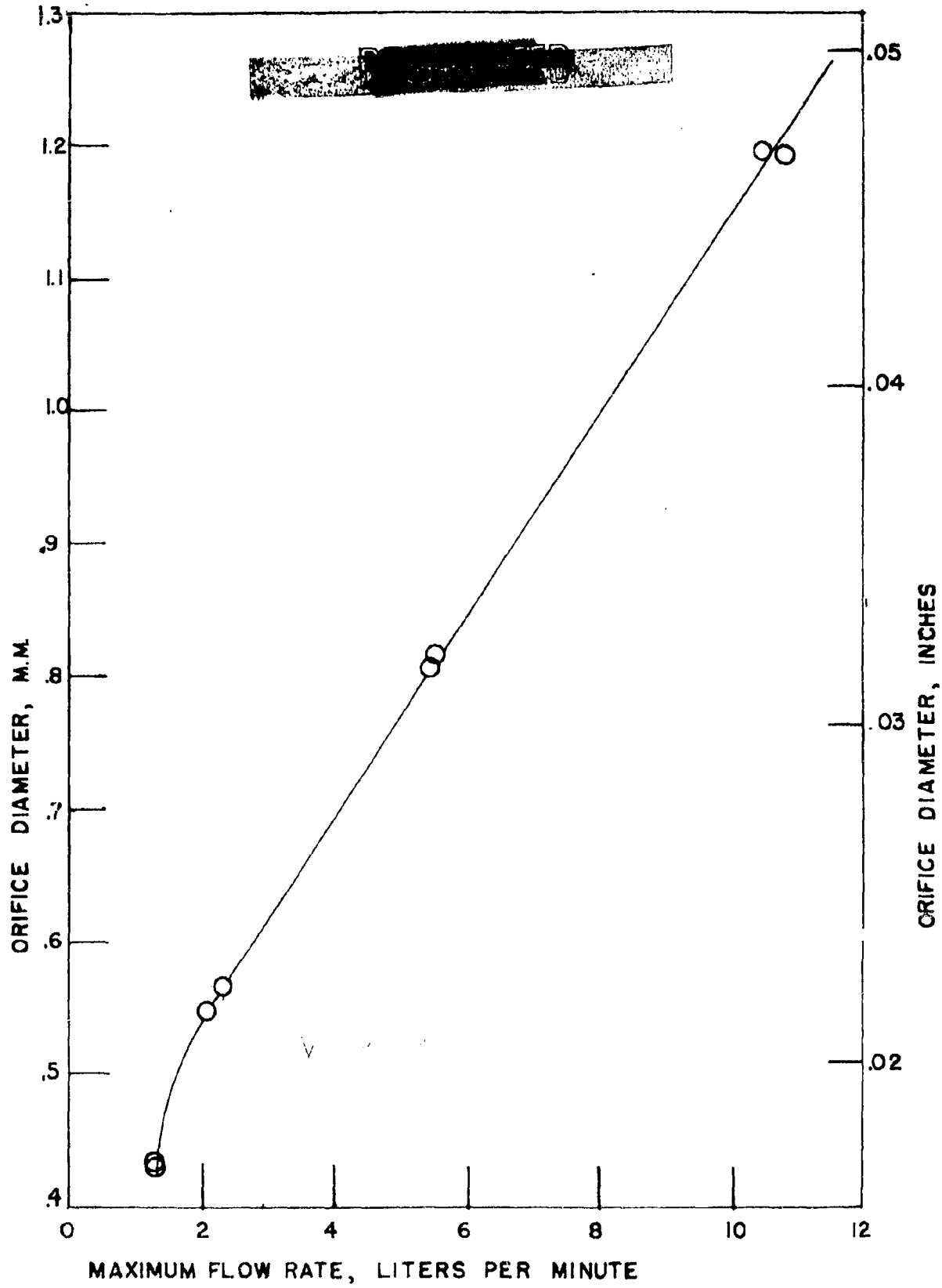

FIGURE 2.- MAXIMUM FLOW RATE VERSUS DIAMETER OF IMPINGER ORIFICE.

Maximum or limiting flows are obtained when the pressure downstream from the orifice is 0.53 (approximately half) or less of the upstream pressure [22]. The velocity of the air through the orifice under these conditions is the maximum, that is, equal to the speed of sound; hence the name sonic-speed for the impinger. This limiting-flow phenomenon eliminates the necessity for a flowmeter, thus simplifying the sampler. The intake tube is placed in a rubber stopper that holds the end of the capillary tube or orifice about 5 mm. above the floor of the flask. The tip of the orifice dips into 25 ml. of an appropriate collecting liquid. Distilled water is commonly used in the collection of stable material as phenol red (used as a dye tracer) and the diluting liquid (used in making serial dilutions for plate counts) in the collection of biological materials. A few (about four) drops of olive oil may be used to control foaming of the impinger liquid.

The mechanism of collection of particulate matter by the impinger is not understood fully. The high-velocity air stream from the orifice apparently: (1) Blows the body of the liquid away from the floor of the flask directly under the orifice, (2) atomizes some of the nearby liquid, (3) causes the particles and liquid droplets, from atomization, to impinge against the floor of the flask or against liquid, and (4) may cause the particles to become charged. Efficiency of collection has been found to be about the same for such varied conditions as: (1) Location of the orifice 20 mm. above the top of the quiet liquid level, (2) location of the orifice so that the air stream is directed parallel to the floor of the flask, and (3) location of the orifice in its usual position, 5 mm. above the floor of the flask. Efficient collection when the orifice is located 20 mm. above the level of the quiet liquid seems to indicate that washing or bubbling through the liquid is not an important part of the collection process. The efficiency in sampling viable organisms decreased when the orifice was located 1 mm. from the floor of the flask, possibly because the air leaving the orifice kept water away from the small space under the orifice and some organisms collected in this space were destroyed by the passage of relatively dry air over them.

III. STUDY OF IMPINGER

The studies made on the impinger were: (1) Determination of the portion of the collected material retained in the intake tube of the impinger, (2) study of the effect of the impinging distance or distance between the bottom of the orifice and the floor of the flask on the sampling efficiency, (3) effect of sampling flow rates on the sampling efficiency, and (4) sampling efficiency.

[22] Perry, J.H., 1941, Chemical Engineers' Handbook: McGraw-Hill Book Co., Inc., New York, 2d Ed., 3029 pp. See p. 847.

Aerosols containing phenol-red particles, Bacillus globigii spores, or Serratia marcescens alone or in combinations were used in these studies. These aerosols were prepared by continuous, constant dissemination of liquid preparations of these materials into the air flowing through a modified Reyneirs Germ-Free chamber 23/.

The liquid was sprayed at rates near 0.9 ml. per minute by a peripheral-air jet atomizer 24/ into 100 liters of air per minute entering the 340-liter chamber. Thus the material from 0.9 ml. of liquid was dispersed in 100 liters of air. The concentration of sprayed material or agent in the resulting aerosol was calculated from the formula:

$$S = \frac{L \times M}{V} \quad \text{- - - - - - - - - - - - - - - - - (2)}$$

in which S = calculated (not actual) concentration of sprayed material in the air in units per liter of air, L = rate of spraying liquid into the air in ml. per minute, M = concentration of agent in liquid sprayed in units per ml., and V = air flow, liters per minute, into which liquid is sprayed. This value, S, is hereafter referred to as the calculated concentration.

The calculated concentration is used in calculating recovery, which is defined for this report as the ratio, expressed in per cent, of the determined to the calculated concentration. The formula used for calculation of recovery is:

$$R = 100\ C'/S \quad \text{- - - - - - - - - - - - - - - - - (3)}$$

in which R = recovery in per cent, C' = determined concentration, as in equation (1), and S = calculated concentration, as in equation (2).

The size of the particles, but not of the spores or the bacteria, was varied by changing the concentration of one of the constituents (usually dextrin) of the liquid disseminated. The size varied approximately with the concentration of material in the liquid.

Particle size was determined by a cascade-impactor 25/ 26/ procedure. Samples of the aerosols were drawn through cascade impactors containing four nozzles of decreasing size, a dry impinger, and a glass-wool filter. Fractions of the particulate matter of decreasing size were removed on adhesive-coated slides behind the nozzles, in the adhesive-coated dry impinger, and in the glass-wool filter. The masses of material in each

23/ Reyneirs, J.A., 1943, Micrurgical and Germ-Free Techniques; Their Application to Experimental Biology and Medicine: C.C. Thomas, Baltimore, Md. 274 pp.

24/ Rosebury, T., et al, See footnote 1.

25/ Green, H.L., and Stevenson, J.W., 1944, Instructions for the Use of the Cascade Impactor: Porton Rept. No. 1600, Camp Detrick Tech. Library . . No. 3782.

26/ May, K.R., 1945, The Cascade Impactor, An Instrument for Sampling Coarse Aerosols: J. Sci. Instruments (London), 22, pp. 187-195.

[redacted]

size fractions were then determined by a suitable procedure. The cumulative per cents of the total of all material collected in the different fractions were plotted on logarithmic-probability paper against the previously determined average size of material collected by the particular jet or impinger. The mass median diameter, or MMD, is the 50-per cent value on such curves. This value is the diameter of the particle at the mid-point of the mass distribution; half the mass is in particles having larger diameters and the other half in particles with smaller diameters. The MMD's give no information on the distribution or variation in size, but where the distributions are similar, as they apparently are in the particles from the atomizers used, they are important values for comparison. Distribution is shown by the shapes and slopes of the logarithmic-probability curves. Phenol red in the samples was determined by colorimetric procedures and microorganisms by standard bacteriological counting techniques.

A. Retention of Collected Material in the Intake Tube. Retention of collected material in the intake tube of the impinger during the quantitating procedure was investigated as a possible cause of significantly low results. Impinger samples of S. marcescens and of B. globigii spores were used in these studies. The intake tubes of the impingers used for collecting these samples were washed carefully with 10 ml. portions of gelatin diluent. The numbers of organisms recovered in these portions were compared with the numbers recovered in 25 ml. of the same medium in the impinger. Table 1 contains information on the results of this study.

These results indicate that the portion of collected organisms retained in the intake tube of the impinger during the quantitating procedure is too small to have any significant effect on the results.

Recent tests with phenol red have indicated that only about 0.1 per cent of this material is retained in the intake tube. Thus these results are in general agreement with the preceding ones on organisms.

B. Effect of Impinging Distance on Sampling Efficiency. The effect of impinging distance, or of the distance between the tip of the orifice and the floor of the impinger flasks on sampling efficiency, was studied. Samples of phenol red and of S. marcescens were collected from the air by impingers having the usual 5 mm. impinging distance and other impinging distances. The usual 25 ml. of liquid were used in all impingers. The depth of this amount of liquid in the 125 ml. impinger flask is 7 mm.

Table 2 contains information on this study.

These results suggest that increasing the impinging distance to at least 28 mm. in collecting phenol red and to 45 mm. in collecting S. marcescens has no significant effect on the sampling efficiency or final results.

Reduction of the impinging distance to 1 mm. or less in collecting S. marcescens apparently caused a reduction in the sampling efficiency, as discussed in the section on description of the impinger.

[redacted]

[redacted]

TABLE 1

Retention of Collected Material in the Intake Tubes of Impingers

Material sprayed and sampled (1)	Impinger number (2)	Calculated total number of organisms recovered from: 25 ml. gelatin diluent in impinger (3)	Calculated total number of organisms recovered from: 10 ml. gelatin diluent washings from intake tube (4)	Organisms recovered from intake tube, per cent of total organisms (5)
S marcescens in distilled water	50	4.7×10^6	1.1×10^3	0.023
	60	4.4×10^6	1.6×10^2	.004
	65	4.9×10^6	3.0×10^2	.0006
	67	5.4×10^6	3.3×10^2	.006
S. marcescens in 3% gelatin	52	2.8×10^7	5.9×10^3	0.021
	58	3.5×10^7	6.9×10^3	.020
	66	3.5×10^7	1.0×10^4	.030
	69	3.7×10^7	1.4×10^4	.014
B. globigii spores in distilled water	13	2.5×10^6	1.7×10^3	0.069
	59	3.5×10^6	2.8×10^3	.080
	67	2.8×10^6	2.8×10^3	.10
	68	2.8×10^6	1.6×10^3	.057
	71	2.7×10^6	2.7×10^2	.010
	78	2.3×10^6	9.5×10^2	.040

[redacted]

TABLE 2

Effect of Impinging Distance on Sampling Efficiency

Material sprayed and sampled (1)	Calculated concentration, gamma per liter of air (2)	Impinging distance or distance from orifice to floor of flask, mm. (3)	Concentration, number of organisms or gamma of material per liter of air (4)	Recovery, or determined/ calculated concentration, per cent. (100 x col. 4/col. 2) (5)
0.1% phenol red and S. marcescens in distilled water	0.887	5	6.99	78.8
		10	7.08	79.8
0.05% phenol red and S. marcescens in 5% dextrin	0.906	5	5.47	60.4
		18	5.27	58.2
		23	5.36	59.2
		28	5.16	57.0
0.1% phenol red and S. marcescens in 5% dextrin	0.906	5	4.76	52.5
		10	4.79	52.8
		13	4.87	53.7
		18	4.75	52.4
S. marcescens in distilled water	—	5	3.7×10^6	—
			3.1×10^6	
			4.5×10^6	
			Ave. = 3.8×10^6	
"	—	45	3.3×10^6	—
"			3.4×10^6	
"			3.8×10^6	
			Ave. = 3.5×10^6	
	—	1	2.7×10^6	—
"			2.7×10^6	
"			3.6×10^6	
"			Ave. = 3.0×10^6	
S. marcescens in distilled water	—	5	1.5×10^6	—
			1.4×10^6	
			Ave. = 1.5×10^6	
	Less than 1		1.1×10^6	—
			1.0×10^6	
			Ave. = 1.1×10^6	

The preceding results are in general agreement with those showing that variation of the impinging distance in the Greenburg-Smith impinger over the range of 2 to 12 mm. had no significant effect on the sampling efficiency of this impinger for dusts and fumes. [27]

C. Effect of Sampling Rates on Sampling Efficiency. The sampling efficiency of the Greenburg-Smith impinger for dusts and fumes increases with sampling rates up to the maximum flow rate [28]. The sampling efficiency of the present impinger was studied to determine whether its efficiency varies in a similar way. Information on this variation was obtained from samples of phenol red, B. globigii, and S. marcescens particulates collected by impingers at different sampling rates up to a maximum flow rate of 10 liters per minute. Such impingers were used instead of the usual ones having maximum flow rates of about 2.5 liters per minute because the 10-liter-per-minute ones were about the smallest ones for which flows as low as 10 per cent of the maximum would be practical from the standpoints of orifice diameter and size of sample. The sampling rate or per cent of maximum flow for maximum sampling efficiency, at least for the 10-liter-per-minute impingers, was determined in this study. In addition, the efficiencies of impingers having maximum flows ranging from 1.3 to 16.5 liters per minute were measured at maximum flow, using phenol red, B. globigii, and S. marcescens.

Table 3 contains information on this study of the effect of sampling rates up to the maximum flow rate on the sampling efficiency of impingers having maximum flows of 10 liters per minute.

Two types of aerosols were used in this study. Both types were prepared by spraying a liquid suspension, at the rate of 0.9 ml. per minute, into 100 liters per minute of air entering the 340-liter Reyniers-chamber apparatus. The suspensions used for one type consisted of distilled water containing 0.1 per cent phenol red and heat-shocked B. globigii spores. The suspensions used for the other type of aerosols consisted of 2 per cent gelatin containing S. marcescens.

Sets of two simultaneous samples were collected for 10 minutes from each aerosol by impingers operating at 10, 30, 50, 70, and 100 per cent of the 10-liter-per-minute maximum flow. Sampling rates below the maximum were obtained by drawing the maximum flow through secondary orifices located

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27/ Hatch, T., Warren, H., and Drinker, P., 1932, A Modified Form of the Greenburg-Smith Impinger for Field Use, with a Study of its Operating Characteristics: J. Ind. Hyg., 14, pp. 301-311.

28/ Drinker, P., and Hatch, T., 1936, Industrial Dust: McGraw-Hill Book Company, New York, 316 pp. See pp. 112-114.

TABLE 3

Effect of Sampling Rates Up to the Maximum Flow on Sampling Efficiency of Impingers Having Maximum Flow of 10 Liters per Minute

Expt. No.[a] P.r. and B.g.	Expt. No.[a] S.m. only	Sampling rate, per cent of max. flow[b]	Recovery, per cent of calculated concentration: Phenol red[a] Impinger[c]	Phenol red[a] Glass-wool filter[c]	Phenol red[a] Both Sum	Phenol red[a] Both % of sum in impinger	B. globigii[a] Impinger[c]	B. globigii[a] Glass-wool filter[c]	B. globigii[a] Both Sum	B. globigii[a] Both % of sum in impinger	S. marcescens[cd] in impinger	Impinger efficiency, ratio to efficiency at the maximum flow, per cent: Phenol red	B. globigii	S. marcescens
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
11	11	10	45.1	34.6	79.7	56.6	13.8	1.8	15.6	88.5	17.2	47.9	38.4	46.4
12	12	10	36.5	44.5	81.0	45.1	5.7	9.5	15.2	37.5	29.3	41.1	14.6	78.3
13	13	10	25.3	58.1	83.4	30.3	6.9	6.2	13.1	52.7	37.2	24.8	11.5	55.9
—	14	10	—	—	—	—	—	—	—	—	17.6	—	—	91.7
15	15	10	39.5	39.0	78.5	50.3	12.7	7.6	20.3	62.6	36.6	45.2	35.7	98.9
16	—	10	28.7	52.1	80.8	35.5	10.1	10.5	20.6	49.0	—	27.9	12.1	—
17	—	10	37.1	52.4	89.5	41.4	16.9	10.8	27.7	61.0	—	36.0	33.5	—
Average			35.4	46.8	82.2	43.2	11.0	7.7	18.7	58.6	27.6	37.2	24.3	74.2
11	11	30	63.4	21.3	84.7	74.8	23.8	1.8	25.6	93.0	34.7	67.4	66.3	93.5
12	12	30	42.6	30.9	73.5	58.0	22.6	5.2	27.8	81.3	30.2	48.0	57.8	80.7
13	13	30	30.3	52.7	83.0	36.5	13.8	3.5	17.3	78.8	61.5	29.7	23.0	92.5
—	14	30	—	—	—	—	—	—	—	—	23.5	—	—	122
15	15	30	65.1	21.1	84.2	74.9	46.8	6.9	53.7	87.1	45.7	72.2	131	124
16	—	30	54.3	31.9	86.2	63.0	35.6	10.8	46.4	76.7	—	52.7	42.5	—
17	—	30	36.7	50.2	86.9	42.2	16.8	14.0	30.8	54.5	—	35.6	33.3	—
Average			48.4	34.7	83.1	58.2	26.6	7.0	33.6	78.6	39.1	50.9	59.0	102
11	11	50	71.8	13.4	85.2	84.3	32.9	1.1	34.0	96.8	43.7	76.3	91.6	118
12	12	50	67.6	14.3	81.9	82.5	28.5	2.2	30.7	92.8	39.3	76.2	72.9	105
13	13	50	62.4	22.7	85.1	73.3	26.9	1.2	28.1	95.7	68.5	61.2	44.8	103
—	14	50	—	—	—	—	—	—	—	—	29.0	—	—	151
15	15	50	68.1	12.1	80.2	84.9	29.0	2.0	31.0	93.5	49.2	77.9	81.4	133
16	—	50	70.8	23.2	94.0	75.3	28.9	11.1	40.0	72.3	—	68.7	34.5	—
17	—	50	66.0	20.3	86.3	76.5	34.7	1.6	36.3	95.6	—	64.1	68.7	—
Average			67.8	17.7	85.5	79.5	30.2	3.2	33.4	91.1	45.9	70.7	65.6	122

11	11	70	75.7	8.0	81.7	90.2	40.7	1.1	41.8	97.4	49.8	78.3	113	134
12	12	70	75.7	7.9	81.6	90.3	41.9	1.2	43.1	97.2	39.7	83.1	107	106
13	13	70	78.1	12.9	91.0	85.8	34.9	1.7	36.6	95.4	82.5	76.6	58.1	124
—	14	70	—	—	—	—	—	—	—	—	30.9	—	—	151
15	15	70	78.0	7.3	85.3	91.4	36.9	1.5	38.4	96.1	54.3	89.2	104	147
16	—	70	80.1	12.8	92.9	86.2	52.2	2.3	54.5	95.8	—	77.8	62.4	—
17	—	70	77.0	12.4	89.4	86.1	45.7	1.1	46.8	97.6	—	74.7	90.5	—
Average			76.8	10.2	87.0	88.3	42.1	1.5	43.6	96.6	51.4	80.0	89.2	134
11	11	100	94.1	2.7	96.8	97.2	35.9	0.3	35.2	99.2	37.1	100	100	100
12	12	100	88.7	2.4	91.1	97.4	39.1	0.9	40.0	97.8	37.4	100	100	100
13	13	100	102	10.0	112	91.1	60.1	0.2	60.3	99.7	66.5	100	100	100
—	14	100	—	—	—	—	—	—	—	—	19.2	—	—	100
15	15	100	87.4	2.4	89.8	97.3	35.6	0.3	35.9	99.2	37.0	100	100	100
16	—	100	103	2.1	105	98.1	83.7	3.3	87.0	96.2	—	100	100	—
17	—	100	103	3.8	107	96.3	50.5	0.3	50.8	99.4	—	100	100	—
Average			96.4	3.9	100	96.2	50.8	0.9	51.7	98.6	39.4	100	100	100

a/ The phenol red and B. globigii were disseminated from the same suspension in each run. These suspensions were prepared from distilled water, 0.1 per cent phenol red, and heat-shocked B. globigii spores. The numbers x 10^7 of spores per ml. of suspension in experiments 11-17 were: 3.6, 3.8, 3.0, 2.3, 2.2, and 3.1 respectively. Spraying rate was 0.9 ml. per minute into 100 liters of air per minute. Temperatures during the runs were within the range 25-26°C. Relative humidities in the runs were: 50, 40, 46, 34, 46, and 44 per cent respectively.

b/ Sampling times were 10 minutes in all runs.

c/ The experimental values in these columns are the averages of the results of the determinations.

d/ The S. marcescens was disseminated from suspensions containing 2 per cent gelatin. The numbers x 10^8 of the S. marcescens per ml. of suspension in experiments 11-15 were 5.3, 3.4, 3.4, 4.2, and 2.6 respectively. Disseminating rate was 0.9 ml. into 100 liters of air per minute as in preceding experiments. Temperatures were within the range 27-28 1/2° C. Relative humidities in the experiments were: 38, 52, 59, 40, and 38 per cent respectively.

downstream from the samplers. Each impinger, used for collecting samples of phenol red and spores of B. globigii from the same aerosol, was followed by a glass-wool filter to collect material passing through the impinger. The phenol red and spores of B. globigii were determined in each sample. Impingers, used for collecting samples of S. marcescens, were not followed by filter samplers because of the low sampling (not collecting) efficiency of such filters for vegetative organisms.

Evaporation of impinger liquid by passage of air through it for 10 minutes has been found to be about 0.05 (of the 25) ml. at 1 liter per minute and 1.5 ml. at the maximum flow of 10 liters per minute. Results were not corrected for this evaporation loss. Resulting errors probably were small compared to other ones.

The recoveries of the different agents by the impinger at the different sampling rates are listed in Table 3 in columns 4, 8, and 12. Figure 3 contains curves showing the relation between the sampling rates and the averages of these recoveries. The recoveries for both the phenol red and B. globigii spores increased with sampling rates up to the maximum flow rate as does the sampling efficiency of the Greenburg-Smith impinger for dust and fumes [29].

The recoveries of S. marcescens increased with sampling rates up to at least 70 per cent of maximum flow and then for some reason, possibly related to destruction of these fragile organisms by impact or passage of large quantities of air over them, the recoveries decreased with further increase in sampling rate.

Impinger efficiencies or ratios, expressed in per cent, of the preceding recoveries to the corresponding recoveries at maximum flow are listed in columns 13-15. Figure 4 shows how these efficiencies varied with the sampling rate.

The sums of the impinger and filter recoveries are listed in columns 6 and 10. The averages of these values increased with sampling rates. The increase for phenol red was slow with rates up to at least 70 per cent of maximum but was rapid for higher rates. The increase for the spores of B. globigii was somewhat erratic but apparently greater than for the phenol red. The reasons for these increases are not known but may have been related to increase in the evaporation of impinger liquid with sampling rate.

Impinger efficiencies, or ratios of impinger recovery (columns 4 and 8) to the sum of the recoveries determined by both the impinger and filter (columns 6 and 10), are listed in columns 7 and 11. Figure 5 contains curves showing the relation between the sampling rates and these efficiencies. These curves show the same general trends as those in Figure 3 for impinger recoveries.

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[29] Drinker, P., and Hatch, T., See footnote 28.

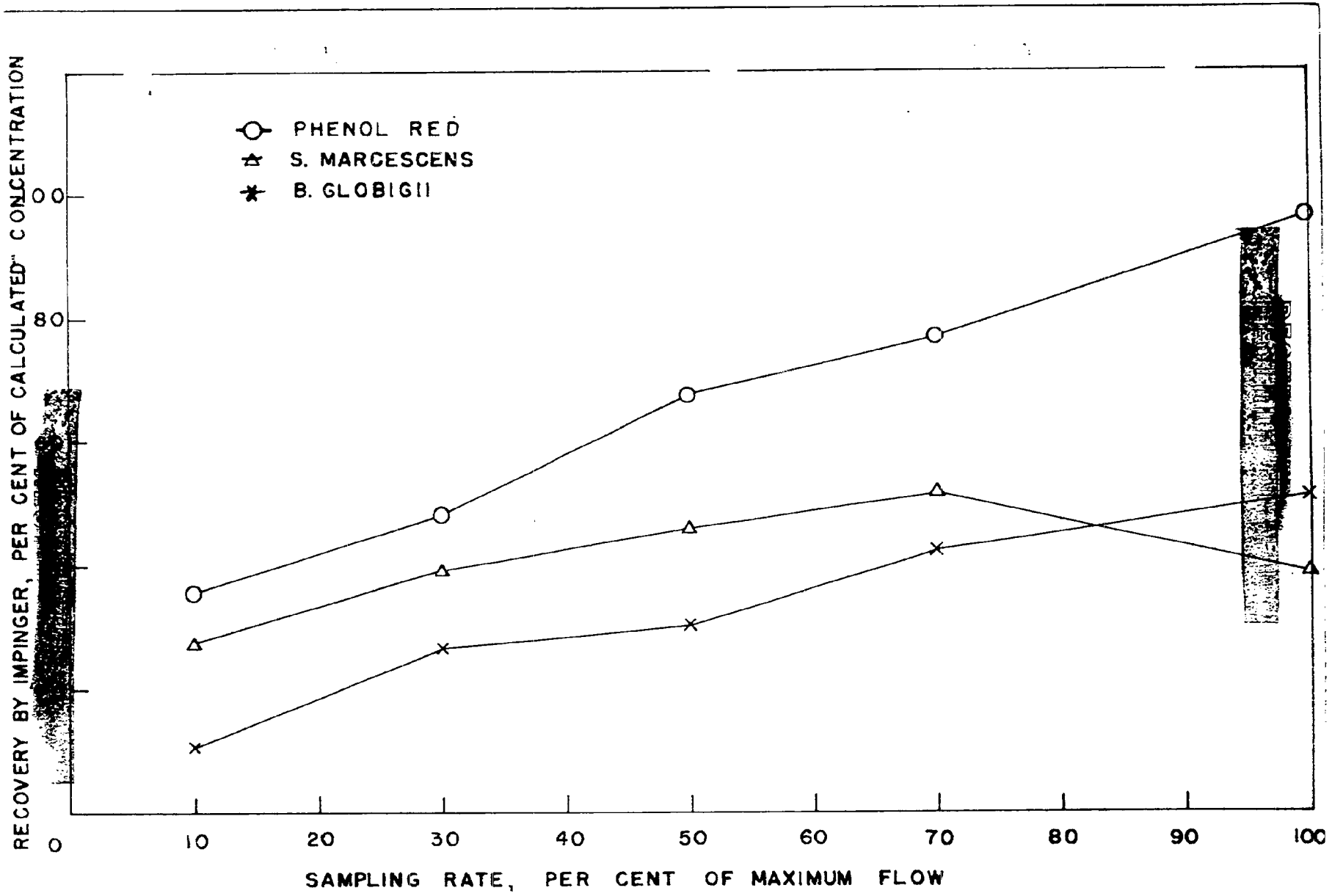

FIGURE 3.- IMPINGER RECOVERY VERSUS SAMPLING RATES UP TO THE MAXIMUM FLOW OF TEN LITERS PER MINUTE.

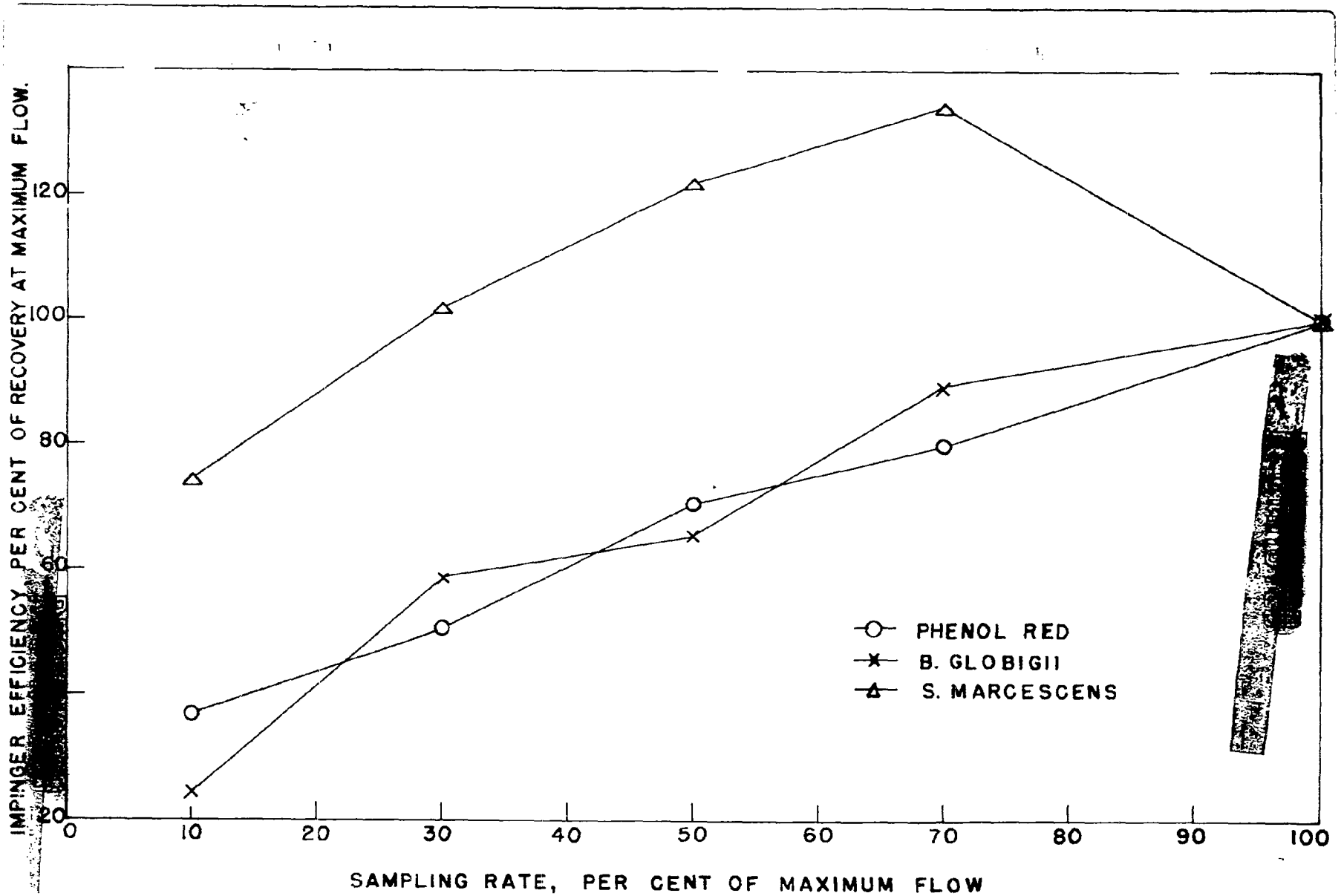

FIGURE 4.- IMPINGER EFFICIENCY, PER CENT OF RECOVERY AT MAXIMUM FLOW VERSUS PER CENT OF MAXIMUM FLOW.

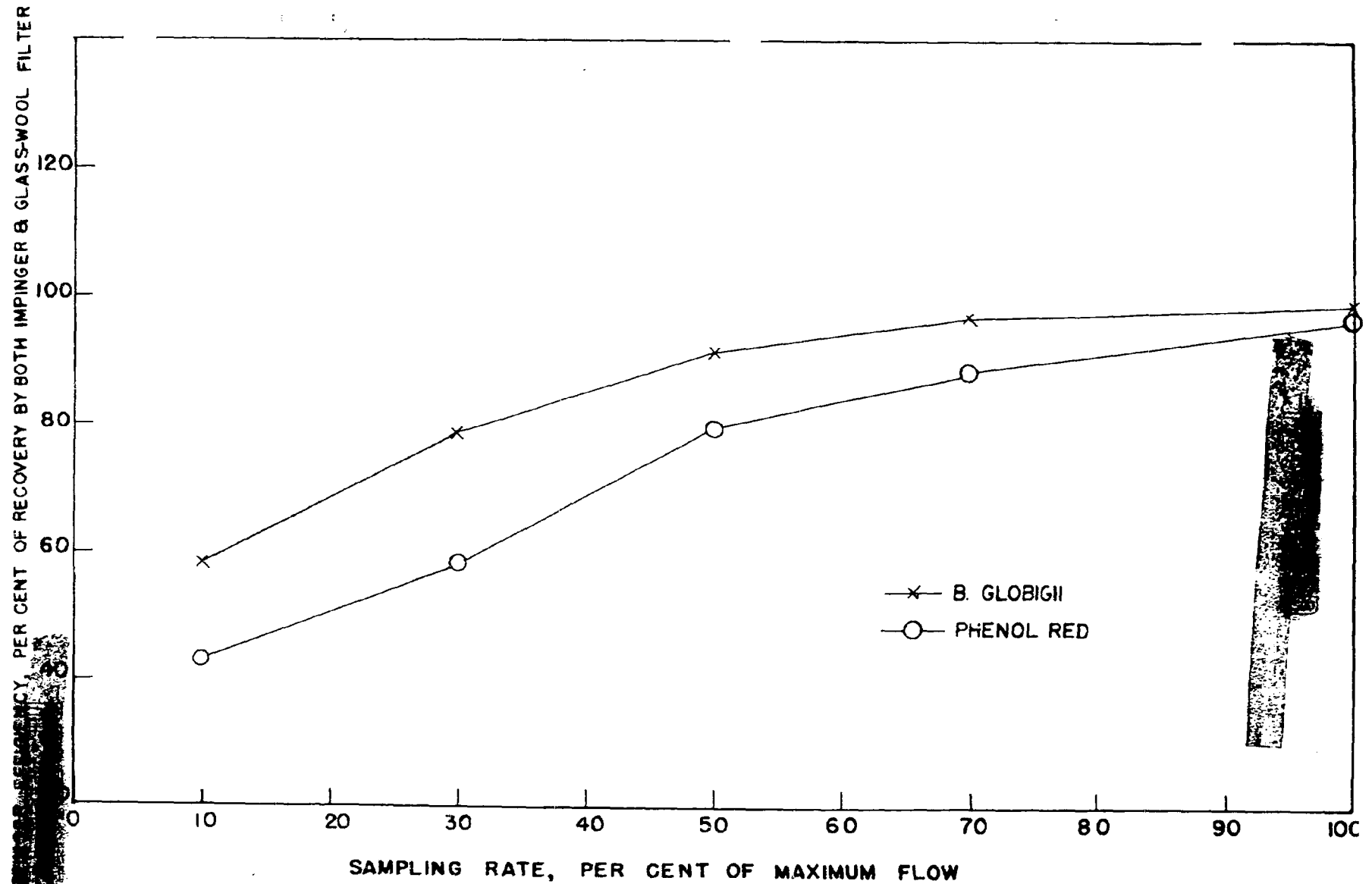

FIGURE 5.- IMPINGER EFFICIENCY VERSUS FLOWS UP TO THE MAXIMUM FROM SERIES — SAMPLING STUDIES.

The preceding results indicate that the sampling efficiency of impingers with a maximum flow of 10 liters per minute for phenol red and spores of B. globigii increases with sampling rate up to the maximum flow rate and that the efficiency for S. marcescens also increases with the sampling rate up to at least 70 per cent of the maximum flow. The decrease in sampling efficiency for S. marcescens at higher sampling rates probably is not important enough to justify sampling at 70 per cent of maximum flow and using a flow meter rather than sampling at the maximum flow where the use of a flow meter is unnecessary.

A study was made to determine over what range of maximum flow rates a high sampling efficiency is obtained. Impingers with orifices for maximum flows in the five following ranges: (a) 1.3 to 1.9, (b) 2.2 to 2.6, (c) 4.9 to 5.4, (d) 10.1 to 10.6, and (e) 16.4 to 16.5 liters per minute were used in this study to collect samples of phenol red, B. globigii and S. marcescens. Each agent was sprayed separately. Two sets of samples of phenol red and one of B. globigii and of S. marcescens were collected. Pairs of simultaneous samples, with one exception, were collected of each material at each sampling range for 10 minutes. One sample instead of a pair was collected in the exception.

Table 4 and Figure 6 contain information on the results of this study. The constancy of the values for S. marcescens in this study over the whole sampling range, and the fair constancy of the values for phenol red and B. globigii, at least up to sampling rates of 10 liters per minute indicate that high sampling efficiencies are obtained at maximum flow rates at least up to 10 liters per minute and probably up to 16 liters per minute.

D. Sampling Efficiency. Some information on sampling efficiency has been given in the preceding studies described in this report. More information, obtained by material-balance and series-sampling procedures, is given in this section. In the material-balance procedure, the amount of material determined in the air by the impinger was compared with the difference between the total amount of material sprayed into the air and the amount deposited on chamber surfaces. In the series-sampling procedure, the amount of material found in the air by the impinger, followed in series by another efficient sampler, was compared with the total amount of material determined by both samplers.

1. Material-balance Studies. Table 5 contains information on these studies. Phenol red, the material sampled, was sprayed from distilled water containing 0.1 per cent phenol red and from 0 to 10 per cent dextrin (column 2), used to vary the size of the resulting particles (column 4). Figure 7 shows the variation of the size with the dextrin concentration in the different experiments. The amount of material deposited on chamber surfaces during the impinger sampling period was determined, in two experiments, from all the deposited material washed from the chamber surfaces into 2 liters of distilled water (column 5) and, in the other three experiments, from material deposited on horizontal or horizontal and vertical microscope slides (columns 6 and 7). The per cent of material deposited on the surfaces varied with the size of the particles. Figure 8 contains information on this variation. The calculated per cent of material in the air during

[redacted]

TABLE 4

Sampling Efficiencies at Various Maximum Flow Rates

Material sprayed and sampled (1)	Calculated concentration, gamma of material or no. of organisms per liter of air (2)	Maximum flow rate, liters per minute (3)	Impinger number (4)	Recovery, per cent: Individual value (5)	Recovery, per cent: Average (6)
Phenol red in distilled water	11.7	1.90	B4	72.6	
		1.86	B5	84.6	78.6
		2.56	96	73.5	
		2.57	98	73.5	73.5
		4.91	C4	72.6	
		5.38	C5	73.5	73.1
		10.58	A4	73.5	
		10.37	A5	72.6	73.1
		16.40	L4	65.0	
		16.48	L11	67.5	66.3
Phenol red in distilled water	9.8	1.90	B4	81.2	
		1.86	B5	82.2	81.7
		2.56	96	82.2	
		2.57	98	89.3	85.8
		4.91	C4	83.2	
		5.38	C5	85.3	84.3
		10.58	A4	82.2	
		10.37	A5	80.2	81.2
		16.40	L4	73.1	
		16.48	L11	73.1	73.1

[redacted]

TABLE 4 (Cont.)

Sampling Efficiencies at Various Maximum Flow Rates

Material sprayed and sampled (1)	Calculated concentration, gamma of material or no. of organisms per liter of air (2)	Maximum flow rate, liters per minute (3)	Impinger number (4)	Recovery, per cent: Individual value (5)	Recovery, per cent: Average (6)
B. globigii in distilled water	5.15×10^5	1.39	C	83.5	
		1.33	G	89.3	86.4
		2.32	62	71.8	
		2.18	84	87.4	79.6
		5.09	C6	77.7	77.7
		10.37	A5	77.7	
		10.06	A6	75.7	76.7
		16.40	L4	66.0	
		16.48	L11	64.1	65.0
S. marcescens in 2% gelatin	2.0×10^7	1.90	B4	17.6	
		1.86	B5	13.1	15.4
		2.32	62	15.1	
		2.18	84	13.6	14.4
		4.91	C4	14.1	
		5.38	C5	17.1	15.6
		10.58	A4	13.6	
		10.37	A5	15.1	14.4
		16.40	L4	15.1	
		16.48	L11	16.6	15.9

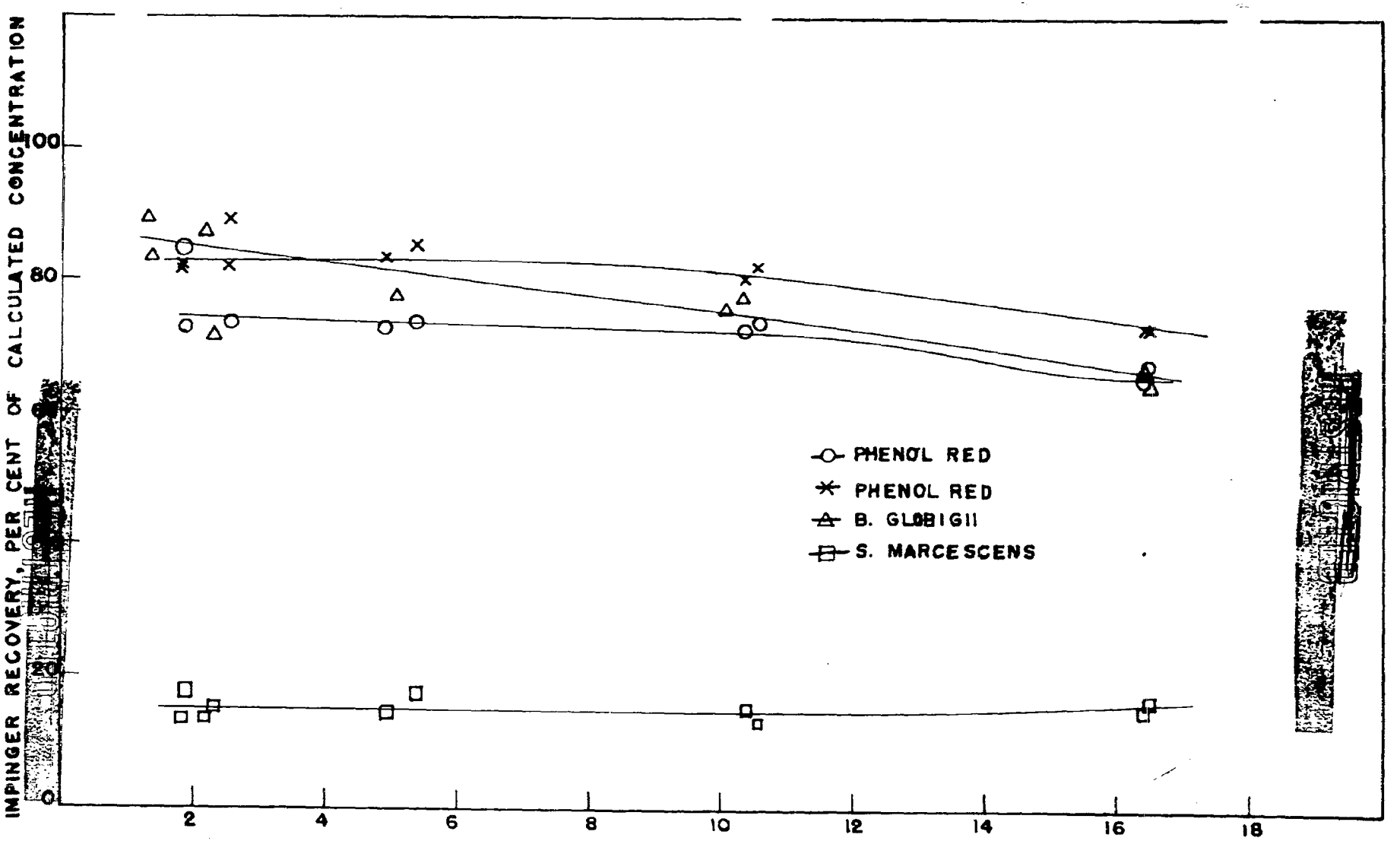

FIGURE 6.- IMPINGER RECOVERY VERSUS MAXIMUM FLOW RATE.

TABLE 5

Sampling Efficiency of Impingers as Determined by Material-balance Studies

Experiment No. (1)	Per cent of dextrin in suspension containing 0.1% phenol red (2)	Phenol red disseminated, γ per liter of air (3)	Size of aerosol particles, MMD in μ (4)	Recovery of phenol red, per cent: Deposited on chamber surfaces: By washing surfaces (5)	By collecting on slides: Horizontal (6)	By collecting on slides: Vertical (7)	In Air: Calculated (100-deposited) (8)	In Air: Recovered by impinger (9)	Sampling efficiency of impinger (columns 9/8 x 100) (10)
4	0	13.5	—	9.9	—	—	90.1	87.7 a/	97.3
6	0	10.6	—	18.7	—	—	81.3	86.0 a/	105.8
26	0	8.80	0.65	—	8.9	—	91.1	94.2	103.4
27	3	8.80	2.15	—	26.9	—	73.1	76.9	105.2
28	5	8.80	2.41	—	31.0	—	69.0	73.5	106.5
29	10	8.80	2.67	—	37.5	C	62.5	57.0	91.2
22	1	8.80	1.30	—	25.2	—	74.8	81.0	108.3
23	3	8.80	1.55	—	29.3	—	70.7	70.8	100.1
24	5	8.80	1.64	—	38.8	—	61.2	64.5	105.4
25	10	8.80	2.17	—	42.3	—	57.7	56.4	97.7
34	1	8.80	1.38	—	18.8	3.7	77.5	84.8	109.4
35	3	8.80	1.96	—	27.2	5.8	67.0	75.1	112.1
36	5	8.80	2.08	—	32.0	3.0	65.0	68.3	105.1
37	10	8.80	2.25	—	38.4	9.3	52.3	63.2	120.8
								Average =	104.9

a/ These results were obtained from several impinger samples collected during the spraying period. Information on these samples follows:

Expt. No.	Air flow through chamber, liters/ minute	Sampling time, min. from start of spraying	Phenol red found in 2.5 l/min. impinger,
4	90	0-5	2,630
		5-10	4,550
		10-15	5,050
		15-20	5,300
		20-25	3,330
		25-30	665
		Total	21,525
6	90	0-4	1,248
		4-8	2,640
		8-12	3,095
		12-16	3,450
		16-20	3,310
		20-24	1,915
		24-28	547
		28-32	160
		Total	16,365

All other results are averages of results of two impinger samples collected for 10 minutes during the equilibrium period at chamber concentration.

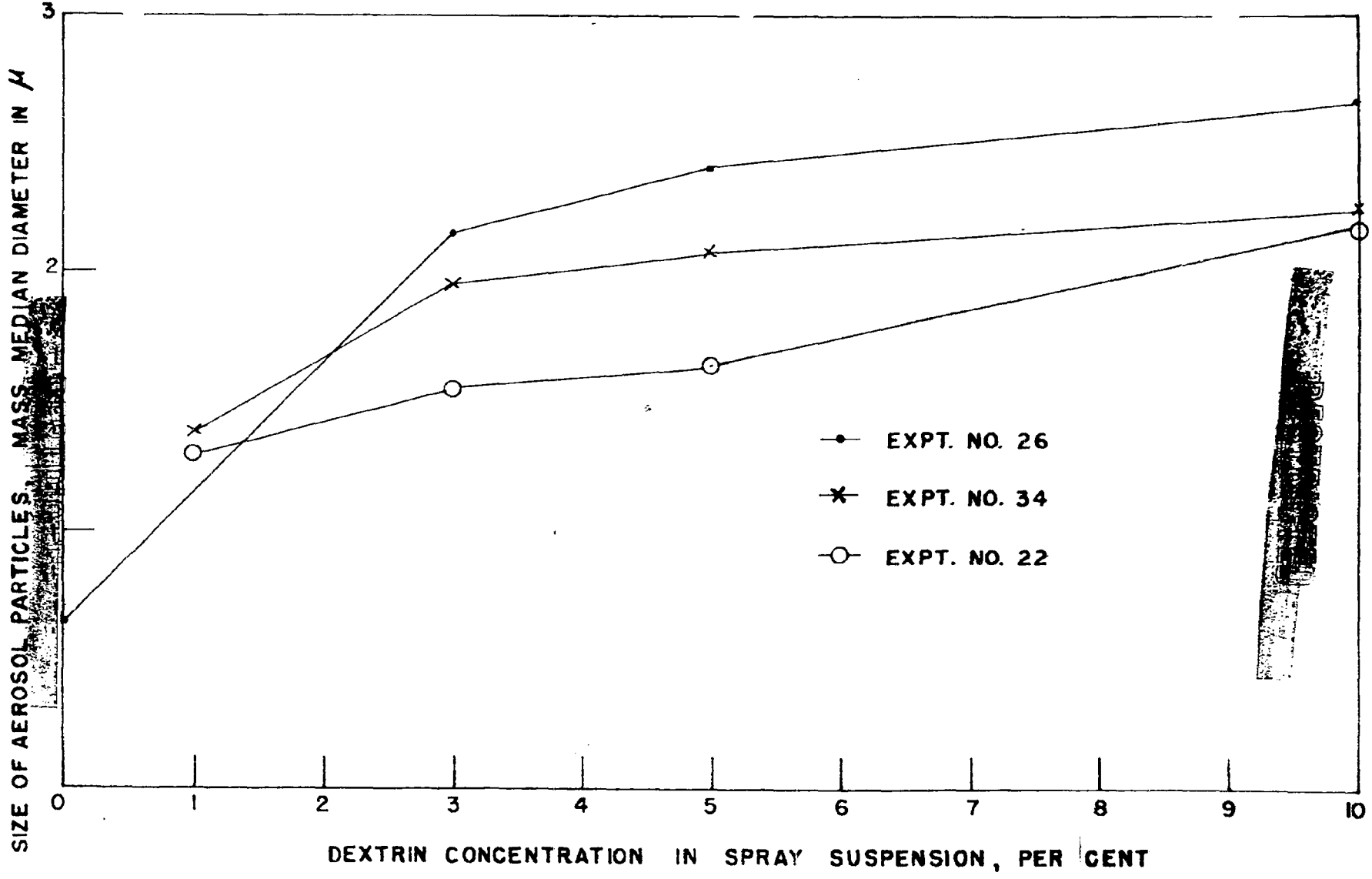

FIGURE 7.- SIZE OF AEROSOL PARTICLES VERSUS PER CENT OF DEXTRIN IN THE SUSPENSION SPRAYED.

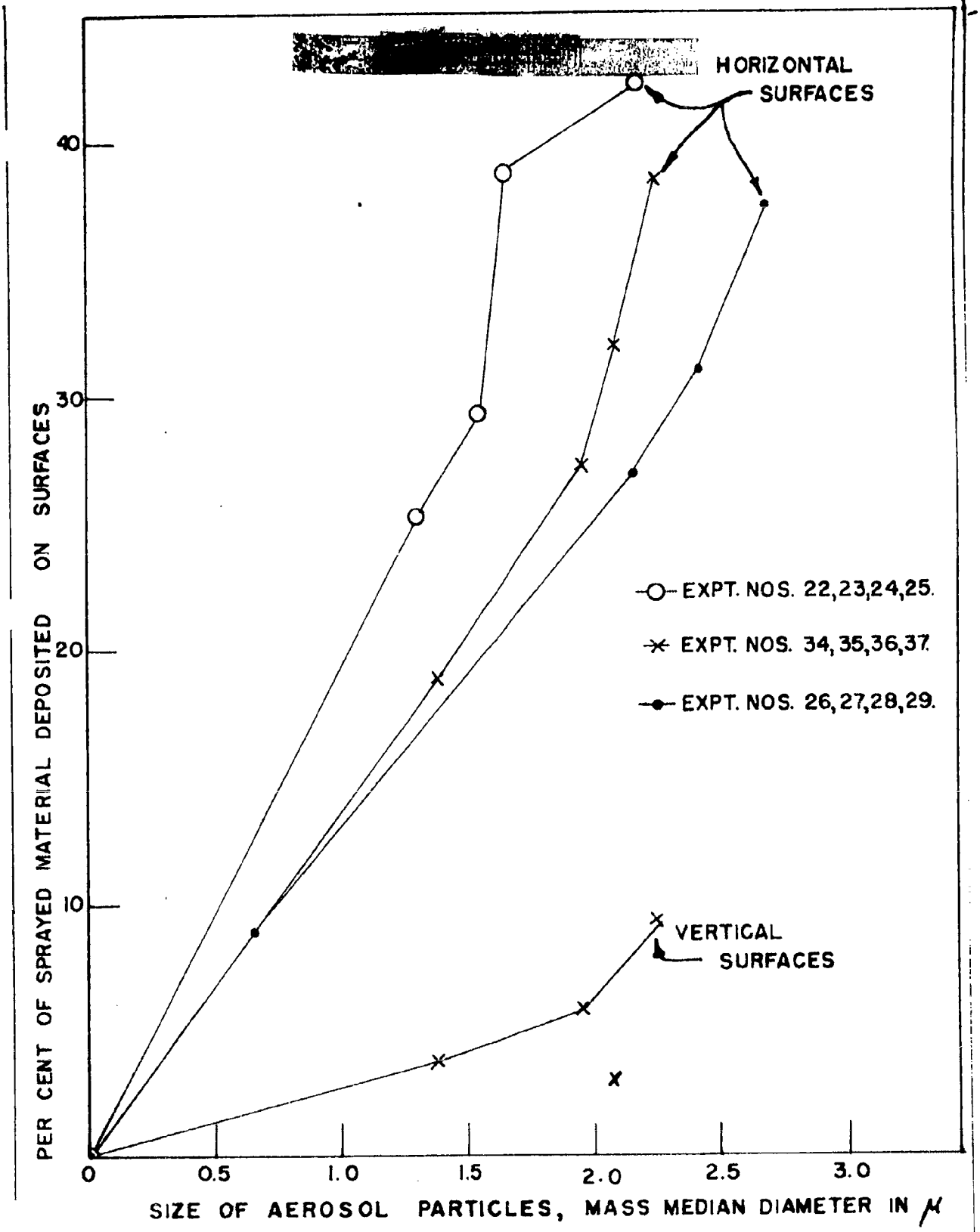

FIGURE 8.- AMOUNT OF MATERIAL DEPOSITED ON SURFACES VERSUS SIZE OF AEROSOL PARTICLES

the impinger sampling period was determined by subtracting the per cent of deposited material from 100 (column 8). The concentration of phenol red in the air determined by the impinger (column 9) was compared with the calculated concentration to determine the sampling efficiency of the impinger. A material balance between the amount of phenol red sprayed and the amounts deposited on surfaces and present in the air was obtained in this procedure. The sampling efficiencies ranged from 91.2 to 120.8 per cent and had an average of 104.9 per cent. Thus, this absolute method indicates that the impinger has a very high sampling efficiency for such particles. The efficiencies apparently did not vary significantly with the size of the particles (MMD's from 0.65 to 2.67 μ) sampled.

2. Series-sampling Studies. Table 6 contains information on these studies. Phenol red, B. globigii, and S. marcescens were the materials used. The phenol red and B. globigii were sprayed from the same liquids; distilled water and 5 per cent dextrin (column 5). The particles from the 5 per cent dextrin suspension probably were much larger than those from the distilled water as shown by Figure 7. S. marcescens was sprayed from 5 per cent dextrin. Impingers with maximum flow rates of 2.5, 5, or 10 liters per minute, followed in series by glass-wool-filter samplers, were the sampling combinations used in most tests (columns 2 and 3). Glass-wool-filter samplers followed in series by 2.5-liter-per-minute impingers and Moulton-Puck-Lemon atomizing-bubbler samplers, followed in series by 5 liter-per-minute impingers, were combinations used in a few tests.

The concentrations of sprayed material determined by the first samplers in the series are listed in column 6. The values in each set (experiments 11-17, 8, and 9) are, with few exceptions, reasonably constant. Some of the variation probably resulted from changes in the conditions of the aerosol. The value for the Moulton sampler in consecutive experiment 11 (column 6) is lower than the other values in this set, possibly because of a low sampling efficiency of this sampler for the small particles. The corresponding value for the Moulton sampler, in sampling the larger particles from the 5 per cent dextrin suspension (consecutive experiment 16), is in fair agreement with the values from the other samplers. The value for the glass-wool-filter sampler in sampling B. globigii from a distilled-water suspension (consecutive experiment 26, column 6) is a little lower than the other values in the set, possibly not because of low collecting efficiency but because of retention of the collected organisms by the filter in the washing process. The value for the glass-wool-filter sampler in sampling S. marcescens from a 5-per cent dextrin suspension (consecutive experiment 36, column 6) is much lower than the other values in the set, possibly for the same reason, that is, retention (or destruction) of the collected organisms by the glass-wool-filter.

All but one of the sampling efficiencies of the first sampler in each series (column 8) were well over 90 per cent. The low value, 83 per cent, is the one for the sampling efficiency of the Moulton sampler for phenol red in small particles (consecutive experiment 11, column 8).

These high sampling efficiencies are in agreement with those obtained in the preceding material-balance studies and with those obtained in studies on

TABLE 6

Sampling Efficiency of Impingers as Determined by Series-Sampling Studies

Experiment Number		Samplers in series		Material sprayed and sampled	Concentration of sampled material, per liter of air	Recovery, per cent		Sampling efficiency of first sampler (col. 7/8), per cent
Consecutive (1)	Actual (2)	First (3)	Second (4)	(5)	(6)	By first sampler (7)	By both samplers (8)	(9)
1	11	Impinger, 2.5 l/min.	Glass-wool filter	0.1% phenol red and B. globigii in distilled water	9.4	91.6	92.1	99.5
2	12	"	"	"	9.4	88.2	91.6	96.3
3	13	"	"	"	9.4	93.1	98.8	94.2
4	15	"	"	"	9.4	83.8	89.9	93.3
5	16	"	"	"	9.4	98.9	105.3	93.9
6	17	"	"	"	9.4	92.7	97.0	95.6
7	8	Imp.-10 l/min	Glass-wool filter	"	9.4	76.6	78.7	97.3
8		" -5 l/min	"	"		78.7	80.3	98.0
9		" -2.5 "	"	"		74.0	76.1	97.2
10		Glass-wool filter	Imp.-2.5 l/min.	"		73.4	75.4	97.3
11		Moulton	" -5 l/min	"		57.4	69.2	83.0
12	9	Imp-10 l/min.	Glass-wool filter	0.1% phenol red and B. globigii in 5% dextrin	9.4	58.8	58.8	100
13		" -5 "	"	"		62.1	63.1	98.4
14		" -2.5"	"	"		62.2	63.2	98.4
15		Glass-wool filter	Imp.-2.5 l/min.	"		56.9	57.0	99.8
16		Moulton	" -5 l/min.	"		54.0	54.0	100

17	11	Imp. -2.5 l/ min.	Glass-wool filter	B. globigii and 0.1% phenol red in distilled water	3.4×10^5	41.2	41.4	99.5
18	12	" "	"	"	3.6×10^5	47.2	47.5	99.4
19	13	" "	"	"	2.8×10^5	52.9	53.1	99.6
20	15	" "	"	"	2.2×10^5	50.0	51.1	97.8
21	16	" "	"	"	2.1×10^5	61.2	61.7	99.2
22	17	" "	"	"	2.9×10^5	57.0	57.3	99.5
23	8	" -10"	"	"	3.3×10^5	26.7	27.0	98.9
24		" -5 "	"	"		35.8	36.1	99.2
25		" -2.5"	"	"		38.9	39.1	99.5
26		Glass-wool filter	Imp.-2.5 l/min.	"		19.5	20.2	96.4
27		Moulton	" -5 l/min	"		38.0	39.0	97.5
28	9	Imp. -10 l/min.	Glass-wool filter	B. globigii and 0.1% phenol red in 5% dextrin	3.6×10^5	28.8	28.9	99.7
29		" -5 l/min	"	"		20.6	20.7	99.5
30		"-2.5 "	"	"		22.5	22.6	99.6
31		Glass-wool filter	Imp.-2.5 l/min.			24.4	24.5	99.5
32		Moulton	" -5 l/min.	"		38.3	38.4	99.6
33	2	Imp. -10 l/min.	Glass-wool filter	S. marcescens in 5% dextrin	8.5×10^5	11.9	—	—
34		" -5 l/min	"	"		18.9	—	—
35		"-2.5 "	"	"		15.7	—	—
36		Glass-wool filter	Imp. -2.5 l/min.	"		6.5	—	—
37		Moulton	"-5 l/min	"		13.2	—	—

[redacted]

the sampling efficiencies of various impingers for dust and fumes 30/ 31/ 32/.

30/ Drinker, P., and Hatch, T., See footnote 28.

31/ Littlefield, J.B., Feicht, F.L., and Schrenk, H.H., 1938, Efficiency of Impingers for Collecting Lead Dusts and Fumes: U.S. Bureau of Mines Rept. of Investigations 3401, 9 pp.

32/ Lodingham, J.M., 1943, The Efficiencies of Impingers and Fibre-Glass Filters in the Sampling of Particulate Clouds: B.D.P. Rept. 31, (Porton). Camp Detrick Tech. Library No. 3783.

[redacted]

[redacted]

IV. SUMMARY

A sonic-speed, liquid impinger used ~~by the Aerobiology Branch~~ in collecting samples of particulate matter (containing such materials as bacteria, dye tracers, toxins, and viruses) from aerosols is described.

This sampler consists of a 125-ml. filtering flask containing an intake tube held in place by a rubber stopper with the lower end of the tube 5 mm. from the floor of the flask. The intake tube is a tube 1 cm. in diameter having a piece of capillary tubing sealed to its lower end. The diameter of the capillary tube or orifice is such that the maximum flow, or the flow at which the velocity of air through the orifice is the same as that of sound, is the desired sampling rate. Twenty-five ml. of a liquid suitable for holding the particulate matter to be sampled is used in the impinger.

The portion of the collected material retained in the intake tube during the usual quantitating procedure was found to be too small to justify a special removal procedure.

Variation of the impinging distance over wide limits (from 2 to 45 mm.) was found to have no significant effect on the sampling efficiency.

Sampling efficiency, at least for phenol red and spores of B. globigii, was found to increase with flow up to the maximum. High sampling efficiencies were found at maximum flows ranging from 1 to 16 liters per minute.

Sampling efficiency was found to be high (well over 90 per cent) by material-balance and series-sampling procedures.

[redacted]

V. RESPONSIBILITY

This report was prepared by C.E. O'Bryon.

Preliminary drafts of this report by T. Rosebury, M. Boldt, and E.L. Neff, Jr. and of a review of samplers by R.E. Porter were used in the preparation of this report.

Assistance in the editing of this report was given by C.E. Brown.

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